Dietary intake of n−3 and n−6 fatty acids and the risk of clinical depression in women: a 10-y prospective follow-up study[1–4]

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ABSTRACT

Background: The associations between different sources of dietary n−3 (omega-3) and n−6 (omega-6) fatty acids and the risk of depression have not been prospectively studied.

Objective: The objective was to examine the relation between different n−3 and n−6 types with clinical depression incidence.

Design: We prospectively studied 54,632 US women from the Nurses' Health Study who were 50–77 y of age and free from depressive symptoms at baseline. Information on diet was obtained from validated food-frequency questionnaires. Clinical depression was defined as reporting both physician-diagnosed depression and regular antidepressant medication use.

Results: During 10 y of follow-up (1996–2006), 2823 incident cases of depression were documented. Intake of long-chain n−3 fatty acids from fish was not associated with depression risk [relative risk (RR) for 0.3-g/d increment: 0.99; 95% CI: 0.88, 1.10], whereas α-linolenic acid (ALA) intake was inversely associated with depression risk (multivariate RR for 0.5-g/d increment: 0.82; 95% CI: 0.71, 0.94). The inverse association between ALA and depression was stronger in women with low linoleic acid (LA) intake (*P* for interaction = 0.02): a 0.5-g/d increment in ALA was inversely associated with depression in the first, second, and third LA quintiles [RR (95% CI): 0.57 (0.37, 0.87), 0.62 (0.41, 0.93), and 0.68 (0.47, 0.96), respectively] but not in the fourth and fifth quintiles.

Conclusions: The results of this large longitudinal study do not support a protective effect of long-chain n−3 from fish on depression risk. Although these data support the hypothesis that higher ALA and lower LA intakes reduce depression risk, this relation warrants further investigation.   *Am J Clin Nutr* 2011;93:1337–43.

INTRODUCTION

Major depressive disorder (MDD) is a chronic and recurrent illness that affects 2 times as many women as men (1). In the United States, ≈1 in 5 women will be affected during their lifetime (2, 3). Lowered intake of n−3 relative to n−6 fatty acids has been implicated in the pathogenesis of depression (4, 5). Substantial evidence from randomized controlled trials indicates that long-chain n−3 from fish is associated with improvement of depressive symptoms (6, 7). However, the associations between different sources of dietary n−3 and n−6 fatty acids, and their ratio, and the risk of depression have not been prospectively studied.

Linoleic acid (LA) and α-linolenic acid (ALA), which represent ≈88.5% (≈15 g/d) and ≈8.5% (≈1.5 g/d), respectively, of polyunsaturated fatty acid (PUFA) intake in the United States, are the primary sources of fatty acids in human tissues (8, 9). Because they require the same metabolic enzymes to form derivative long-chain n−6 and n−3 fatty acids, experimental studies have raised concerns that LA intake may reduce ALA incorporation in tissues and/or its conversion to long-chain n−3 (10–12). Therefore, a potential competition effect of LA may be more applicable to ALA than to long-chain n−3 intake [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)].

To date, the link between n−3 fatty acids intake and the risk of developing new-onset depression has been analyzed in only 3 prospective cohorts (13–15). Results have been inconsistent, perhaps due to a number of limitations in the study designs such as the following: use of a single dietary intake assessment at baseline, small sample size, definition of depression, and short follow-up. We therefore accessed data from the Nurses' Health Study to examine the relation between different n−3 and n−6 fatty acids and the risk of incident clinical depression. Our analysis improved on previous work in terms of sample size, repeated measurements of dietary intake, adjustment for updated lifestyle risk factors, and a more rigorous definition of clinical depression.

SUBJECTS AND METHODS

Study population

Established in 1976, the Nurses' Health Study is a prospective cohort of 121,700 registered female nurses aged 30–55 y at enrollment and residing in 11 states of the United States. The

cohort has been contacted every 2 y with mailed questionnaires about lifestyle and medical history, which update exposure information and inquire about newly diagnosed medical illnesses. The analysis excluded women who left ≥11 food items blank in food-frequency questionnaires, reported implausible energy intake (<600 or >3500 kcal/d), or did not return or answer the long-form questionnaires in 1996, 1998, or 2000. Of the 76,516 remaining women, we sequentially excluded those who reported physician-diagnosed depression in 1996 ($n = 4286$) or with an unknown start date ($n = 168$), those who reported taking regular antidepressant medication in 1996 ($n = 2636$), and those who presented severe depressive symptoms in 1992 ($n = 2799$) or 1996 ($n = 2381$) [ie, score of ≤52 on the Mental Health Index (MHI-5), a 5-item subscale of the Short-Form 36 Health Status Survey (16, 17)]. A lower score (≤52) on the MHI-5 has been associated with high sensitivity and specificity for MDD (18). After excluding those who had missing values for exposure variables ($n = 9614$), the final 1996 baseline population comprised 54,632 women. The Institutional Review Boards of Brigham and Women's Hospital and the Harvard School of Public Health approved the study protocol.

Dietary assessment

In 1980, a 61-item food-frequency questionnaire containing a single question on fish intake was administered to assess the intake of specific fats and other nutrients (19). In 1984, this food-frequency questionnaire was revised to incorporate questions on 116 individual foods that included 4 fish and seafood items (dark-meat fish, canned tuna, other fish and shellfish). Food-frequency questionnaire reproducibility and validity (20), and calculation of intake of EPA+DHA fatty acids have been reported elsewhere (21). Because the subsequent questionnaires contained food items that were critical for the assessment of n−3 and n−6 intake, we considered the 1984 questionnaire as the starting point for dietary information. These expanded questionnaires incorporate additional questions regarding types of fats used in cooking and the brand of margarine. Questions were also asked about frequency of utilization of mayonnaise or other creamy salad dressing and oil and vinegar (eg, Italian) salad dressing. Salad dressing and mayonnaise fatty acid compositions have been imputed from soybean oil, which accounted for all (salad dressing) or most (mayonnaise) of the market until recently. Composition values for ALA and other nutrients were obtained from the Harvard University Food Composition Database, which is derived from US Department of Agriculture sources (22), and complemented by direct analysis of food samples obtained from Boston area grocery stores and fast-food restaurants. More details have been published elsewhere (23). Food contributors to the overall intake of ALA were also previously published (24).

All nutrient intakes were adjusted for total energy intake according to the residual model (25). As a proxy measure of long-term dietary exposure, we took the cumulative average of the 4 dietary assessments (1984, 1986, 1990, and 1994) before our baseline (1996). This average better represents the long-term dietary intakes than does a single baseline assessment and is less likely to be affected by reverse causation than the more recent dietary intake. Information on fish-oil consumption was available only in 1990 and 1994.

Case ascertainment

Clinical depression was defined as reporting both physician-diagnosed depression and regular antidepressant medication use. Because questions relating to clinical depression were first addressed in 1996, we considered that year's questionnaire cycle as the baseline for follow-up. The question of regular antidepressant medication use was first asked in 1996, and this information was updated biennially through 2006. In 2000, nurses were asked to report the year of their first physician-diagnosed depression (1996 or before, 1997, 1998, 1999, or 2000). Thereafter, this information was updated biennially through 2006. Deaths were identified from the National Death Index, by next of kin, or by the postal system. Combining all sources, we estimated that follow-up for deaths was >98% complete (26).

Statistical analysis

Among women who were free of significant depressive symptoms at baseline, person-years of follow-up were calculated from the date of return of the 1996 questionnaire to the first endpoint—death, 1 June 2006, or the date of return of their last questionnaire, whichever came first. The age-standardized baseline characteristics of participants according to quintiles of ALA and EPA+DHA are presented for descriptive purposes (**Table 1**). Cox proportional hazards models, stratified on age in months and questionnaire cycle, served to estimate the relative risks (RRs) and their 95% CIs of developing clinical depression. The choice of units to express the RRs for continuous n−3 and n−6 intakes was based on differences between the 90th and 10th percentiles of their cumulative average intakes. Multivariate analyses were adjusted for nondietary covariates, including hormonal status (postmenopausal without and with hormonal therapy, premenopausal); ethnicity (white, yes or no); obesity [body mass index (BMI; in kg/m^2) ≥30, yes or no]; smoking status (never smoked; past smoker; currently smoke 1–14 cigarettes/d, 15–24 cigarettes/d, or ≥25 cigarettes/d); physical activity (quintiles); reported diagnosis of diabetes, cancer, or myocardial infarction or angina; and multivitamin use (yes or no) (model 1). All nondietary covariates were updated biennially. We further adjusted for cumulative average intake of dietary covariates (all continuous), including energy (kcal/d), alcohol (g/d), protein (g/d), *trans* fatty acids (g/d), saturated fatty acids (g/d), monounsaturated fatty acids (g/d), other n−3 and n−6 PUFAs (g/d), and fish-oil consumption (never, 1990 only, 1994 only, or both 1990 and 1994) (model 2). In sensitivity analysis (model 3), 691 cases of clinical depression observed before June 1998 were excluded. We performed further sensitivity analyses with the exclusion of 11,462 women who reported general medical conditions that could cause mood symptoms according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (27).

We also calculated the cumulative average of total fish intake from 1984 through 1994. Multivariate Cox proportional hazards models were also adopted to estimate the RRs (95% CIs) of developing clinical depression according to fish consumption frequency. Participants were classified into 5 groups: <1 time/mo (reference group), 1–3 times/mo, 1 time/wk, 2–4 times/wk, and ≥5 times/wk.

Interaction between ALA and LA intake was assessed by using a cross-product term, with both elements as continuous variables.

TABLE 1
Baseline (1996) characteristics of participants in the Nurses' Health Study according to quintiles (Q) of n−3 intake[1]

Variable	Quintiles of ALA intake (median g/d)					Quintiles of EPA+DHA intake (median g/d)				
	Q1 (0.75)	Q2 (0.86)	Q3 (0.94)	Q4 (1.04)	Q5 (1.20)	Q1 (0.08)	Q2 (0.13)	Q3 (0.18)	Q4 (0.25)	Q5 (0.37)
n	10,975	10,907	10,877	10,957	10,916	10,862	10,923	11,061	10,873	10,913
Age (y)	63.1 ± 7.1[2]	62.7 ± 7.1	62.6 ± 7.0	62.8 ± 7.0	63.0 ± 7.0	62.3 ± 7.3	62.3 ± 7.1	62.7 ± 7.0	63.1 ± 6.9	63.8 ± 6.6
General medical conditions (%)[3]	21.3	21.7	20.9	20.7	20.5	21.0	21.2	21.2	20.7	21.0
White (%)	96.5	97.8	98.1	98.4	98.4	98.7	98.8	98.3	97.4	96.1
Hormonal status (%)										
Postmenopausal, no hormones	30.6	29.8	30.3	29.7	30.6	32.2	31.7	29.6	28.5	29.1
Postmenopausal, with hormones	59.6	60.0	60.3	60.8	60.0	57.8	58.6	61.0	61.8	61.1
Premenopausal	9.8	10.2	9.4	9.5	9.4	10.0	9.7	9.4	9.7	9.8
Reported diagnosis of (%)										
Myocardial infarction or angina	3.8	4.0	3.5	3.8	3.5	3.4	3.6	3.7	3.8	4.1
Diabetes	3.6	4.4	4.5	4.4	5.0	4.3	4.1	4.3	4.6	4.5
Cancer	7.0	6.0	6.3	6.3	6.3	5.7	6.3	6.5	6.9	6.6
BMI (in kg/m² ≥30 (%))	16.1	18.7	19.3	20.9	22.1	18.4	18.4	19.3	20.0	21.1
Physical activity (MET-h/wk)	19.4 ± 23.9	18.5 ± 22.0	18.7 ± 21.1	18.3 ± 21.7	18.0 ± 21.1	15.7 ± 21.6	17.0 ± 20.0	18.3 ± 20.7	19.6 ± 22.2	22.4 ± 24.7
Current smoking (%)	10.0	10.2	10.4	10.6	12.2	12.2	11.6	10.5	10.2	8.9
Aspirin use (≥7/wk) (%)	16.5	16.0	15.4	15.8	15.6	15.8	15.8	16.1	15.7	16.0
Multivitamin use (%)	55.3	54.0	52.9	51.0	48.5	49.0	50.4	51.9	53.6	56.8
Fish-oil use (%)	2.8	3.3	3.7	3.9	4.6	2.8	3.1	3.4	4.1	5.0
Daily intake (1984–1994)[4]										
Energy (kcal)	1706 ± 446	1753 ± 440	1786 ± 441	1781 ± 436	1737 ± 449	1754 ± 457	1764 ± 445	1773 ± 449	1763 ± 430	1706 ± 432
Alcohol (g)	6.3 ± 10.6	5.7 ± 8.8	5.6 ± 8.4	5.7 ± 8.3	5.9 ± 8.5	4.7 ± 9.0	5.5 ± 8.8	6.1 ± 9.2	6.4 ± 9.0	6.5 ± 8.7
Vitamin D (IU)	371 ± 210	354 ± 197	347 ± 190	336 ± 188	325 ± 196	295 ± 188	310 ± 183	331 ± 181	362 ± 186	436 ± 211
LA (g)	7.5 ± 1.6	8.3 ± 1.4	9.0 ± 1.4	9.5 ± 1.5	10.9 ± 2.0	9.2 ± 2.1	9.2 ± 1.9	9.1 ± 1.9	9.0 ± 1.9	8.8 ± 1.9
AA (g)	0.13 ± 0.04	0.13 ± 0.04	0.13 ± 0.03	0.13 ± 0.04	0.14 ± 0.04	0.11 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	0.16 ± 0.04
ALA (g)	0.73 ± 0.07	0.86 ± 0.03	0.94 ± 0.02	1.04 ± 0.03	1.24 ± 0.16	0.95 ± 0.20	0.96 ± 0.19	0.96 ± 0.19	0.96 ± 0.19	0.97 ± 0.20
EPA+DHA (g)	0.21 ± 0.14	0.21 ± 0.13	0.21 ± 0.13	0.21 ± 0.13	0.21 ± 0.14	0.07 ± 0.02	0.13 ± 0.01	0.18 ± 0.02	0.25 ± 0.02	0.41 ± 0.13
ALA:LA ratio	0.10 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
n−3:n−6 ratio	0.13 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	0.11 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.17 ± 0.04

[1] All characteristics are age-standardized with the exception of age. Percentages may not total 100% because of rounding and missing data. Intake of vitamin D includes both dietary and supplement sources. AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MET, metabolic equivalents of task.

[2] Mean ± SD (all such values).

[3] Percentages are for the entire follow-up period. General medical conditions associated with mood disorder according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (27): neurological conditions (Parkinson disease, Huntington disease), cerebrovascular condition (stroke), metabolic conditions (pernicious anemia or vitamin B-12 deficiency), endocrine conditions (hyper- and hypothyroidism, hyper- and hypoparathyroidism), autoimmune conditions (systemic lupus erythematosus), and pancreatic carcinoma.

[4] Computed as the cumulative average of 1984 through 1994 (*see* Subjects and Methods). Except for ALA:LA and n−3:n−6 ratios, all nutrient intakes were adjusted for total energy intake with the residual model.

ALA and LA interaction was found to be positively significant for clinical depression risk ($P = 0.02$). Therefore, the association between ALA intake and the risk of clinical depression was also assessed separately within each quintile of LA and vice versa. All analyses were performed with SAS software, version 9.1 (2003; SAS Institute Inc, Cary, NC). All P values reported are 2-sided.

RESULTS

Among 54,632 women who were free from depressive symptoms at baseline, 2823 had experienced incident clinical depression during 10 y of follow-up (495,829 person-years). The age-adjusted baseline characteristics of participants by quintiles of ALA and EPA+DHA intakes are shown in Table 1.

Dietary n−3 intake from plant sources, ALA, was not associated with the risk of depression in age-adjusted or nondietary multivariate models (model 1) (**Table 2**). However, when we further adjusted for dietary factors (model 2), the RR of clinical depression was 0.82 (95% CI: 0.71, 0.94) for each 0.5-g/d increment of ALA. Dietary intake of EPA+DHA from fish (Table 2) or fish consumption frequency (data not shown) was not associated with the risk of clinical depression. Compared with women who ate fish <1 time/mo, RRs (95% CIs) were 0.95 (0.79, 1.15) for those who ate fish 1–3 times/mo, 0.96 (0.81, 1.15) for those who ate fish 1 time/wk, 0.96 (0.78, 1.19) for those who ate fish 2–4 times/wk, and 1.07 (0.74, 1.55) for those who ate fish ≥5 times/wk. When examined separately, neither fatty nor lean fish intake was associated with depression risk. Compared with nonusers of fish-oil supplements, the risk of clinical depression was unexpectedly high in the small fraction of women (n = 689) who reported fish-oil consumption in 1990 only (RR: 1.40; 95% CI: 1.05, 1.87), but no association was found in the 841 women who reported fish-oil use in 1994 only (RR: 1.09; 95% CI: 0.81, 1.46) or in the 475 women who reported use in both 1990 and 1994 (RR: 0.98; 95% CI: 0.64, 1.50) (model 2; data not shown).

The risk of clinical depression increased with increasing LA intake (RR for each 5-g/d increment: 1.26; 95% CI: 1.07, 1.49) (model 2, Table 2) and decreased with increasing ALA:LA ($P <$ 0.001 for trend) and n−3:n−6 ratios ($P = 0.004$ for trend). Arachidonic acid (AA) intake was not significantly associated ($P = 0.80$) with the risk of depression in multivariate model 2. The results obtained after excluding women with general medical conditions were almost identical to those for all women (data not included). When we excluded clinical depression cases observed before June 1998, the results for n−3 and n−6 were similar (model 3, Table 2).

We assessed the multivariable-adjusted risk of clinical depression for ALA and LA intakes within quintiles of LA and ALA, respectively. For each 0.5-g/d increment of ALA, the RR (95% CI) of clinical depression was 0.57 (0.37, 0.87) in the first, 0.62 (0.41, 0.93) in the second, 0.68 (0.47, 0.96) in the third, 0.90 (0.66, 1.23) in the fourth, and 1.09 (0.91, 1.32) in the fifth quintiles of LA (**Figure 1**). For each 5-g/d increment of LA, the RR (95% CI) of clinical depression was 1.04 (0.73, 1.49) in the first, 1.07 (0.71, 1.62) in the second, 1.26 (0.85, 1.86) in the third, 1.71 (1.16, 2.52) in the fourth, and 1.22 (0.93, 1.59) in the fifth quintiles of ALA (**Figure 2**).

DISCUSSION

In this large prospective cohort of women, we found that higher dietary intake of vegetable n−3, ALA, was significantly associated with a lower risk of clinical depression, especially among those who had the lowest intake of LA. We did not observe any association between the risk of clinical depression and fish consumption frequency or EPA+DHA intake from fish. A novel aspect of our study is the more complete analysis of n−3 and n−6 PUFAs and their effect on depression risk and, consequently, the significant interaction noted between LA and ALA. Stratification analyses within quintiles of LA revealed that ALA was associated with a significantly lower risk of clinical depression among women in the first 3 quintiles of LA intake but not among those in the fourth and fifth quintiles (Figure 1).

Because ALA and LA require common metabolic enzymes, when LA intake is low, up to 11.5% of ALA is converted to EPA (10). Therefore, the ability of ALA to slightly raise long-chain n−3, mainly EPA, and to a lesser extent docosapentaenoic acid, might partially explain our results. Evidence from randomized trials indicates that the combination of EPA+DHA improves depressive symptoms when administered as an adjunct to antidepressant medication (6, 7, 28). However, significant heterogeneity between studies and publication bias are noted (7, 28). Some authors have suggested that EPA alone or a higher EPA/DHA ratio is associated with better outcomes than supplementation with DHA alone (6, 29). A protective effect of EPA would be consistent with the inverse correlation that we observed with intake of ALA, of which very little is converted to DHA (no greater than 0.5%) (30). An argument against effects of ALA from its higher conversion to EPA is the absence of relation between EPA+DHA intake and depression in our results. However, EPA intake among our participants was low (median: 58 mg/d) and therefore EPA formation from dietary ALA acid might exceed EPA intake. It is also possible that long-term dietary ALA intake may play a physiologic role in clinical depression that is independent of EPA+DHA.

Despite adjustment for covariates, including ALA, each 5-g increase in LA was associated with a 26% (95% CI: 7%, 49%) higher risk of clinical depression (Table 2). Again, this might be due to the significant interaction between LA and ALA. Stratification analyses within quintiles of ALA indicated that augmented intake of LA was linked with a significantly higher risk of clinical depression only among women in the fourth quintile of ALA (Figure 2). This significantly heightened risk was probably due to chance, because elevated LA intake was not associated with excess risk in the lowest quintiles of ALA (in which the risk might be hypothesized to be the greatest). Therefore, LA might not have a direct detrimental effect on depression but rather a potential biological interaction with ALA. However, an adverse effect of LA intake on risk of depression in susceptible individuals cannot be excluded.

The results of previous longitudinal studies on n−3 intake and the risk of depression have been inconsistent. In a Finnish cohort of 29,133 men aged 50–69 y and followed for 9 y, no associations were noted between dietary intake of EPA+DHA from fish or fish consumption and proxy depression measures (13). This cohort was the only one in which the relation between ALA intake and the risk of depression was analyzed, and no association was found. Among 7903 Spanish participants in the SUN

TABLE 2
Relative risks (RRs) of clinical depression according to n−3 and n−6 intakes in women from the Nurses' Health Study cohort[1]

	Continuous analysis	
	RR (95% CI)	*P* value
ALA (each increase of 0.5 g)		
Age-adjusted[2]	0.99 (0.90, 1.09)	0.86
Multivariate model 1[3]	0.96 (0.87, 1.05)	0.37
Multivariate model 2[4]	0.82 (0.71, 0.94)	0.005
Multivariate model 3[5]	0.81 (0.69, 0.95)	0.009
EPA+DHA (each increase of 0.3 g)		
Age-adjusted[2]	1.05 (0.97, 1.15)	0.21
Multivariate model 1[3]	1.06 (0.97, 1.16)	0.17
Multivariate model 2[4]	0.99 (0.88, 1.10)	0.82
Multivariate model 3[5]	0.96 (0.84, 1.10)	0.57
LA (each increase of 5 g)		
Age-adjusted[2]	1.12 (1.02, 1.23)	0.02
Multivariate model 1[3]	1.07 (0.97, 1.17)	0.19
Multivariate model 2[4]	1.26 (1.07, 1.49)	0.006
Multivariate model 3[5]	1.33 (1.10, 1.61)	0.003
AA (each increase of 0.1 g)		
Age-adjusted[2]	1.22 (1.10, 1.34)	<0.001
Multivariate model 1[3]	1.15 (1.04, 1.27)	0.007
Multivariate model 2[4]	1.02 (0.88, 1.19)	0.80
Multivariate model 3[5]	1.06 (0.89, 1.26)	0.54
ALA:LA (each increase of 0.05 U)		
Age-adjusted[2]	0.81 (0.74, 0.90)	<0.001
Multivariate model 1[3]	0.84 (0.76, 0.92)	<0.001
Multivariate model 2[4]	0.78 (0.69, 0.88)	<0.001
Multivariate model 3[5]	0.77 (0.66, 0.88)	<0.001
n−3:n−6 (each increase of 0.1 U)		
Age-adjusted[2]	0.85 (0.75, 0.98)	0.02
Multivariate model 1[3]	0.89 (0.78, 1.02)	0.10
Multivariate model 2[4]	0.78 (0.66, 0.92)	0.004
Multivariate model 3[5]	0.74 (0.61, 0.90)	0.003

[1] Values are RRs (95% CIs) from Cox proportional hazards models. The RR is for each unit increment of fatty acids or ratios. AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.
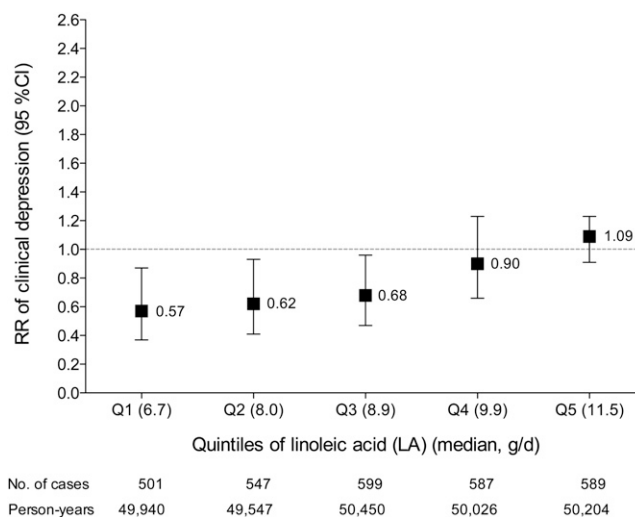
[2] Adjusted for age (continuous) and time interval.

[3] Further adjusted for hormonal status (postmenopausal without or with hormonal therapy, premenopausal), white race (binary), obesity (binary), smoking status (never smoked; past smoker; currently smoke 1–14 cigarettes/d, 15–24 cigarettes/d, or ≥25 cigarettes/d), physical activity (quintiles), reported diagnosis of diabetes (binary), cancer (binary), myocardial infarction or angina (binary), and multivitamin use (binary).

[4] Further adjusted for cumulative average intake (1984–1994) of energy (kcal/d), protein (g/d), *trans* fatty acids (g/d), saturated fatty acids (g/d), monounsaturated fatty acids (g/d), and alcohol (g/d) (all continuous). For ALA, model 2 was further adjusted for EPA+DHA, LA, and AA (all continuous) and fish-oil use (never, 1990 only, 1994 only, both 1990 and 1994). For EPA+DHA, the model was further adjusted for ALA, LA, AA, and fish-oil use. For LA, the model was further adjusted for ALA, EPA+DHA, AA, and fish-oil use. For AA, the model was further adjusted for ALA, EPA+DHA, LA, and fish-oil use. For the ALA:LA ratio, the model was further adjusted for EPA+DHA, AA, and fish-oil use.

[5] Adjusted as in model 2 but excludes cases observed before June 1998 (*n* = 691).

FIGURE 1. Multivariate relative risks (RR; 95% CIs) of clinical depression with increased intake of α-linolenic acid (ALA) according to stratification by quintiles (Q) of linoleic acid (LA). Values are RRs from Cox proportional hazards models. The RR is for each 0.5-g/d increase of ALA. The ALA × LA interaction was significant for clinical depression risk (*P* = 0.02). Multivariate analysis included the following covariates: age (continuous); time interval; hormonal status (postmenopausal without or with hormonal therapy, premenopausal); white race (binary); obesity (binary); smoking status (never smoked; past smoker; currently smoke 1–14 cigarettes/d, 15–24 cigarettes/d, or ≥25 cigarettes/d); physical activity (quintiles); reported diagnosis of diabetes (binary), cancer (binary), or myocardial infarction or angina (binary); multivitamin use (binary); and cumulative average intake (all continuous) of energy (kcal/d), protein (g/d), *trans* fatty acids (g/d), saturated fatty acids (g/d), monounsaturated fatty acids (g/d), alcohol (g/d), eicosapentaenoic acid plus docosahexaenoic acid (g/d), arachidonic acid (g/d), and fish oil (never, 1990 only, 1994 only, or both 1990 and 1994).

(≈67%) were of anxiety disorder. In a US cohort of 3317 men and women, the odds of depressive symptoms were not significantly different in higher quintiles of baseline long-chain n−3 or fish intake after 3 y of follow-up (15). However, an inverse association between long-chain n−3 and the number of visits with depressive symptoms was encountered in women only (15).

The use of 4 dietary assessments over a period of 10 y was a unique strength of our study. Indeed, other cohorts completed only one baseline food-frequency questionnaire as a measure of exposure. This approach is less accurate than ours as it assumes that dietary intake measured once at baseline is representative of usual diet that remains unchanged for the entire follow-up period. In addition, our assessment of ALA and LA has been validated against the amounts of these fatty acids in adipose tissue [*r* = 0.34 (*P* < 0.001) for ALA and *r* = 0.37 (*P* < 0.001) for LA] (23). However, because LA and ALA are largely contributed by the same or similar food items, complete disentangling of LA from ALA intake is not possible, and some degree of misclassification and bias due to residual confounding is inevitable (31). Furthermore, due to the high positive correlation between LA and ALA intake in the US diet, the LA:ALA ratio displays only modest variability; it would thus be of interest to examine the relation between specific fatty acid intakes and depression in populations with dietary patterns that allow better discrimination of ALA from LA. Statistical limitations common to any study with multiple comparisons also apply to the present study.

Two additional concerns are reverse causation and confounding by indication. Reverse causation may derive from an

(Seguimiento Universidad de Navarra) cohort, a lower risk of mental disorder was noted only for the second and fourth quintiles of long-chain n−3 intake after a median follow-up of 27.5 mo compared with the first quintile of intake (14). However, no linear trend was observed, and most of these cases
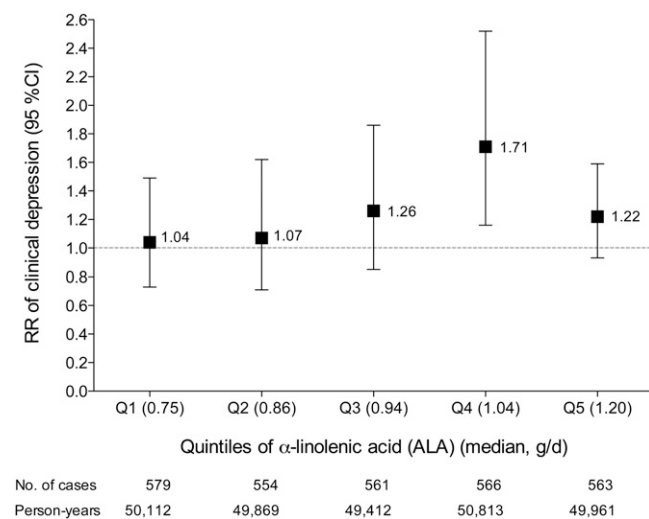
FIGURE 2. Multivariate relative risks (RR; 95% CI) of clinical depression with increased intake of linoleic acid (LA) according to stratification by quintiles (Q) of α-linolenic acid (ALA). Values are RRs from Cox proportional hazards models. The RR is for each 5-g/d increase of LA. The ALA × LA interaction was significant for clinical depression risk (P = 0.02). Multivariate analysis included the following covariates: age (continuous); time interval; hormonal status (postmenopausal without or with hormonal therapy, premenopausal); white race (binary); obesity (binary); smoking status (never smoked; past smoker; currently smoke 1–14 cigarettes/d, 15–24 cigarettes/d, or ≥25 cigarettes/d); physical activity (quintiles); reported diagnosis of diabetes (binary), cancer (binary), or myocardial infarction or angina (binary); multivitamin use (binary); and cumulative average intake (all continuous) of energy (kcal/d), protein (g/d), trans fatty acids (g/d), saturated fatty acids (g/d), monounsaturated fatty acids (g/d), alcohol (g/d), eicosapentaenoic acid plus docosahexaenoic acid (g/d), arachidonic acid (g/d), and fish oil (never, 1990 only, 1994 only, or both 1990 and 1994).

effect of depressive symptoms on diet. For example, a spurious inverse association between n−3 intake and the risk of depression could be observed if women with depressed mood reduced their n−3 intake. To minimize bias from this source, we excluded at baseline 12,270 women with severe depressive symptoms, and we considered the cumulative average of n−3 and n−6 intake assessed between 1984 and 1994 to predict the occurrence of depression between 1996 and 2006. Furthermore, we confirmed the results in sensitivity analyses in which we excluded 691 women with depression before June 1998, and 11,462 women who had general medical conditions associated with mood disorder. Neither of these exclusions altered our findings. Confounding by indication, on the other hand, could obfuscate a true protective effect of n−3 or even induce a false-positive association between the use of fish-oil supplements and the risk of depression. This may happen if women with depressed mood started taking fish oil in an attempt to improve their symptoms, an occurrence that would be consistent with the 40% increased risk noted among the small group of women (1.26%) who consumed fish oil only in 1990. However, considering that no significant risks were discerned for women who used fish oil in 1994 and for those who ingested it in both 1990 and 1994, the heightened risk among such consumers in 1990 only may simply reflect random variation.

Finally, some outcome misclassification is inevitable because of a combination of errors in reporting depression or the intake of antidepressant medications, low recognition of depression by physicians (32), undertreatment of depression (33), and the

prescription of antidepressant medications for indications other than depression. We tried to maximize the specificity of case definition, accepting as incident cases of depression only those women who reported both a diagnosis of depression and the use of antidepressant medications. A significant percentage of diagnosed depressed cases did not receive antidepressant medication during the follow-up. Thus, our strict definition of depression (ie, diagnosis of depression plus use of antidepressant medication) may cause inclusion of only relatively more severe cases. However, we were unable to distinguish between bipolar and unipolar depression. To the extent that the probability of correctly classifying women with an incident case of depression is independent from their dietary habits (nondifferential misclassification of outcome), the low sensitivity of this strict case definition should not bias RR estimates (34). Over 10 y of follow-up, 5.2% of the women in our cohort developed clinical depression. This incidence is not directly comparable to that observed in unselected populations because, to minimize reverse causation, we excluded women with severe depressive symptoms at baseline, thus eliminating a group of women at higher risk of depression. Thus, unlike most previous studies, our analyses of the Nurses' Health Study address the relation between n−3 and n−6 fatty acids and new onset of relatively severe depression—that is, depression severe enough to be diagnosed as such and treated with antidepressant medication.

Several biological mechanisms might potentially explain the effect of ALA in depression. Dietary ALA deficiency has been linked with altered brain biochemistry, such as membrane structure and fluidity, ion channels, second messengers, reduced cyclic AMP response element-binding protein transcription factor activity and brain-derived neurotrophic factor expression, and increased expression of cytosolic and secretory phospholipase A2 and cyclooxygenase-2 (35–37). Animal studies have also indicated that ALA deficiency changes serotoninergic and dopaminergic neurotransmission in the frontal cortex (38, 39), and its augmented intake might influence neurogenesis as well as key proteins involved in synaptic functions (40). Inflammatory processes and endothelial dysfunction are often involved in depression and cardiovascular disease (3). A higher dietary intake of ALA has been related to lower plasma inflammatory biomarkers and endothelial activation among women in the Nurses' Health Study (41). However, the mechanism of action of dietary n−3 and n−6 in depression requires further exploration in humans.

In conclusion, the results of this large longitudinal study do not support a protective effect of long-chain n−3 fatty acids or fish intake on the risk of depression. Higher ALA and lower LA intakes were associated with a reduced risk of clinical depression, but this association should be interpreted cautiously because of the difficulty of separating the effects of correlated nutrient intakes.

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